

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER  
12204-004001

U.S. APPLICATION NO. (If Known, see 37 CFR 1.5)

09/763909

INTERNATIONAL APPLICATION NO.  
PCT/IL99/00464INTERNATIONAL FILING DATE  
25 August 1999PRIORITY DATE CLAIMED  
27 August 1998

## TITLE OF INVENTION

A TRANSCRIPTION FACTOR TFIIID SUBUNIT, TAFII105, POLYPEPTIDES, DNA ENCODING THEREFOR AND PHARMACEUTICAL COMPOSITIONS

## APPLICANT(S) FOR DO/EO/US

Rivka Dikstein and Ayala Yarnit-Hezi

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
  3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
  4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
  5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
    - b. ☒ has been communicated by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
    - b. ☐ have been communicated by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☒ have not been made and will not be made.
  8. ☒ An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
  9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
  10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11 to 16 below concern other documents or information included:**
11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☐ A FIRST preliminary amendment.
  - ☐ A SECOND or SUBSEQUENT preliminary amendment.
  14. ☐ A substitute specification.
  15. ☐ A change of power of attorney and/or address letter.
  16. ☐ Other items or information:

## CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL6883216751/US

I hereby certify under 37 CFR § 1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and addressed to the Commissioner for Patents, Washington, D.C. 20231

February 26, 2001  
Date of Deposit

Signature

Gildardo Vargas  
Typed Name of  
Person Signing

U.S. APPLICATION NO. 09/7763909

INTERNATIONAL APPLICATION NO.  
PCT/IL99/00464ATTORNEY'S CASE NO.  
12204-00400117. ☐ The following fees are submitted:**Basic National Fee ( 37 CFR 1.492(a)(1)-(5) ):**

Neither international preliminary examination fee (37 CFR 1.482)  
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  
and International Search Report not prepared by the EPO or JPO..... **\$1000**

International preliminary examination fee (37 CFR 1.482) not paid to  
USPTO but International Search Report prepared by the EPO or JPO ..... **\$860**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but  
international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **\$710**

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **\$690**

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **\$100**

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$860.00

Surcharge of **\$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$0.00

Claims	Number Filed	Number Extra	Rate	
Total Claims	- 20 =		x \$18	\$0.00
Independent Claims	- 3 =		x \$80	\$0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ \$270	\$0.00

**TOTAL OF ABOVE CALCULATIONS =**

\$860.00

☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are  
reduced by 1/2.

\$430.00

**SUBTOTAL =**

\$430.00

Processing fee of **\$130** for furnishing the English Translation later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(f)).

\$0.00

**TOTAL NATIONAL FEE =**

\$430.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be  
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **\$40.00** per property +

\$0.00

**TOTAL FEES ENCLOSED =**

\$430.00

**Amount to be  
refunded:** \$  
**Charged:** \$

- a. ☒ A check in the amount of \$430.00 to cover the above fees is enclosed.  
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of \$0.00 to cover the above fees. A duplicate  
copy of this sheet is enclosed.  
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive  
(37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

**SEND ALL CORRESPONDENCE TO:**

Gregory P. Einhorn  
FISH & RICHARDSON P.C.  
4350 La Jolla Village Drive, Suite 500  
San Diego, CA 92122  
(858) 678-5070 phone  
(858) 678-5099 facsimile

SIGNATURE:

NAME

REGISTRATION NUMBER

Gregory P. Einhorn

38,440



#3/a

Rec'd PCT/PTO 14 MAY 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Dikstein et al.

Art Unit : Unknown

Serial No. : 09/763,909

Examiner : Unknown

Filed : February 26, 2001

Title : A TRANSCRIPTION FACTOR TFIIID SUBUNIT, TAFII105, POLYPEPTIDES,  
DNA ENCODING THEREFOR AND PHARMACEUTICAL COMPOSITIONS

Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the specification:

Replace the paragraph beginning at page 14, line 10 with the following rewritten paragraph:

-- 5'-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3' (SEQ ID NO: 3)

5'-GATCCGGAAGTCCCCTCGGAAAGTCCCTA-3' (SEQ ID NO: 4)

Mutated NF-kB oligonucleotides:

5'-AGCTTATCTACTTTCCGAGTCTACTTTCCG-3' (SEQ ID NO: 5)

5'-GATCCGGAAGTAGACTCGGAAAGTAGATA-3' (SEQ ID NO: 6) --

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

Date of Deposit

05/10/01

Signature

*Deborah A. Clark*

Typed or Printed Name of Person Signing Certificate

DEBORAH A. CLARK

Applicant : Dikstein et al.  
Serial No. : 09/763,909  
Filed : February 26, 2001  
Page : 2

Attorney's Docket No.: 13005-002001 / 131519.1 JP/bv



REMARKS

Applicant hereby submits that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825 and that no new matter is added. Attached is a marked-up version of the changes being made by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 5/10/01

Gregory P. Einhorn 44,830  
Reg. No. 38,440

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Applicant : Dikstein et al.  
Serial No. : 09/763,909  
Filed : February 26, 2001  
Page : 3

Attorney's Docket No.: 13005-002001 / 131519.1 JP/bv



**Version with markings to show changes made**

**In the specification:**

Paragraph beginning at page 14, line 10 has been amended as follows:

5'-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3' (SEQ ID NO: 3)

5'-GATCCGGAAAGTCCCCTCGGAAAGTCCCTA-3' (SEQ ID NO: 4)

Mutated NF-kB oligonucleotides:

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APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: A TRANSCRIPTION FACTOR TFIID SUBUNIT, TAF<sub>II</sub>105,  
POLYPEPTIDES, DNA ENCODING THEREFOR AND  
PHARMACEUTICAL COMPOSITIONS

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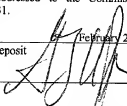
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A TRANSCRIPTION FACTOR TFIID SUBUNIT, TAF<sub>II</sub>105, POLYPEPTIDES, DNA ENCODING THEREFOR AND PHARMACEUTICAL COMPOSITIONS

## 5 FIELD OF THE INVENTION

This invention relates to novel fragments of the transcription factor subunit TAF<sub>II</sub>105 and to DNA molecules encoding them, and to pharmaceutical compositions and therapeutic methods using TAF<sub>II</sub>105 and fragments thereof.

## BACKGROUND OF THE INVENTION

- 10 References referred to in the present specification are listed at the end of the specification before the claims.

- Initiation of transcription is a key regulatory step affecting gene expression in response to a variety of extra- and intracellular signals, during developmental processes and for providing tissue specificity. The rate of
- 15 transcription initiation is determined by proximal and distal enhancer elements that are bound by gene-specific transcription factors (activators or repressors); these are modular in their nature, typically consisting of a DNA binding domain and one or more activation (or repression) domains. The transcription initiation site is determined by a number of general transcription factors (GTFs)
- 20 that assemble around the core promoter to form the preinitiation complex. The general transcription factor TFIID plays an essential role in transcription initiation, as it recognizes and binds the core promoter and nucleates the assembly of the other general initiation factors (TFIIA, TFIIB, TFIIIE, TFIIF,

and TFIIF) and RNA polymerase II (for recent reviews see Hoffman *et al.*, 1997 and Orphanides *et al.*, 1996).

Studies of transcriptional activation mechanisms revealed that TFIID is also required for mediating transcription-activation signals by gene-specific activators (Horikoshi *et al.*, 1988; Pugh and Tjian, 1990). TFIID is a multisubunit complex that consists of the TATA box-binding protein (TBP) and a number of TBP-associated factors (TAFs) that are present in all cells (Verrijzer and Tjian, 1996). Some of these TAFs have been shown to directly bind activation domains of activators. This interaction appears to be essential for activator-dependent transcriptional stimulation *in vitro* as revealed in reconstituted transcription reactions.

A novel TFIID subunit, TAF<sub>II</sub>105, that is highly enriched in TFIID of human and mouse B-lymphocytes, has recently been identified and cloned (Dikstein *et al.*, 1996, and U.S. 5,710,025, whose contents are incorporated herein by reference). TAF<sub>II</sub>105 exists in sub-stoichiometric amounts relative to the core TAFs, consistent with the assumption that a TFIID complex containing this subunit might be required for transcription of a limited set of genes. TAF<sub>II</sub>105 contains two distinct functional domains: a conserved C-terminal domain implicated in TFIID binding and TAF-TAF interactions (putative amino acids 553-802), and an N-terminal coactivator domain (amino acid 1-552) that directs interaction with activation domains of transcription factors. These findings suggest that TAF<sub>II</sub>105 may be a coactivator that mediates activation signals of gene specific activators.

Apoptosis (or programmed cell death) is a natural cellular mechanism for eliminating unwanted cells in an organism. Apoptosis may occur as a result of an external signal, such as the presence of a cytokine, or in response to an internal cellular signal. Pathological conditions involving apoptosis may occur in several variations. On the one hand, undesired apoptosis may occur in healthy cells, while on the other hand, apoptosis may be inhibited in diseased cells, such as cancer cells.



The NF- $\kappa$ B family of transcription factors are important regulators of a large number of biological processes. For example, NF- $\kappa$ B plays an essential role in a variety of immune and inflammatory responses, mediates antigen and cytokine-induced gene expression and is required for cellular response to various external signals. The cytokine TNF- $\alpha$  activates NF- $\kappa$ B by inducing rapid nuclear translocation of NF- $\kappa$ B proteins. Recent studies have revealed that NF- $\kappa$ B protects cells from TNF- $\alpha$  induced apoptosis, presumably by activating anti-apoptotic genes (Beg and Baltimore, 1996; Wang et al., 1996; van Antwerp et al., 1996; Liu et al., 1996). Moreover, activation of anti-apoptotic genes by NF- $\kappa$ B has been shown to be essential for transformation by oncogenic Ras (Finco et al., 1997; Mayo et al., 1997). Likewise, activation of NF- $\kappa$ B by ionizing radiation or daunorubicin was found to protect cells from killing by apoptosis (Wang et al., 1996).

## 15 SUMMARY OF THE INVENTION

The present invention is based on the surprising discovery that TAF<sub>II</sub>105 interacts directly with members of the NF- $\kappa$ B family and mediates gene activation with respect to apoptotic cellular mechanisms. Although TAF<sub>II</sub>105 was previously described, and it was suggested that TAF<sub>II</sub>105 may be responsible for mediating transcription by a subset of activators in B cells, no indication was given that TAF<sub>II</sub>105 might be involved with NF- $\kappa$ B proteins, which function also in non-B cells, nor with the activation of anti-apoptotic genes.

It is an object of the present invention to provide novel polypeptide fragments of the TFIID polypeptide subunit, TAF<sub>II</sub>105, as well as DNA molecules encoding them.

It is a further object of the invention to provide pharmaceutical compositions and methods for use within the framework of a therapy intended to eliminate undesired, pathological cells, e.g. within the framework of cancer therapy.

It is a still further object of the invention to provide pharmaceutical compositions and methods for treating diseases resulting from the pathological occurrence of apoptosis.

In the present specification, the term "*normal biological activity*" of the TAF<sub>II</sub>105 polypeptide refers to mediation by TAF<sub>II</sub>105 of the activation of anti-apoptotic genes by NF- $\kappa$ B, as described hereinafter.

According to one aspect of the present invention, there is provided a DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2, wherein the fragment has a dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105 polypeptide.

Such fragments may be derived from the N- or C-terminus of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2, but preferably they are derived from the N-terminal domain, which is the putative coactivator domain of TAF<sub>II</sub>105. A most preferred fragment consists of the N-terminal amino acids 1 to 552 of TAF<sub>II</sub>105, named herein TAF<sub>II</sub>105 $\Delta$ C. Other preferred fragments contain a sequence of amino acids included within TAF<sub>II</sub>105 $\Delta$ C such as the fragments consisting of the amino acids 1-452, 1-359 and 443-452, or is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids such that the thus obtained modified fragment has a dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2.

Based on the discovery, in accordance with the invention, of the role that TAF<sub>II</sub>105 plays in the apoptotic process, the present invention provides novel therapies allowing, on the one hand, to promote apoptosis of pathological cells and, on the other hand, to prevent undesired apoptosis in normal cells. In the first case, the apoptosis-inhibiting pathway which is mediated by TAF<sub>II</sub>105 is inhibited by neutralizing, directly or indirectly, the TAF<sub>II</sub>105 polypeptide, and in the second case, this apoptosis-inhibiting pathway is enhanced.

In the first aspect of the invention, referred to at times as the "*apoptosis-promoting aspect*", the active TAF<sub>II</sub>105 fragments of the invention

are used to promote and potentiate the apoptotic mechanism, for example in the treatment of cancer, in which case it is desired to stimulate apoptosis in the cancerous cell. Thus, the active TAF<sub>II</sub>105 fragment or a composition containing it may be administered by itself to the patient, or it may be administered together with another chemotherapeutic agent so as to increase its effectiveness.

Thus, according to this aspect of the invention, there is provided a pharmaceutical composition for inducing an apoptotic process in pathological cells, for example in the treatment of cancer, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

- 10 (a) a fragment of the TAF<sub>I</sub>105 polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or more amino acids, wherein the fragment or the modified fragment has a dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105 polypeptide;
- 15 (b) an inhibitor or antagonist of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2;
- (c) a DNA sequence encoding the fragment of (a); and
- (d) a DNA sequence directing expression of an antisense RNA sequence to SEQ.ID. NO.: 1, or a part thereof, and which is
- 20 capable of inhibiting its expression *in vivo*.

Also provided according to this aspect of the invention is the use of an active agent as defined in (a) – (d) above for the preparation of a pharmaceutical composition for use for promoting apoptosis, for example in the treatment of cancer, as well as a method for the promotion of apoptosis, for example in the treatment of cancer, comprising administering to a subject in need, an active agent as defined in (a) – (d) above or a composition comprising such agent.

In the second aspect of the invention, referred to at times as the “apoptosis-preventing aspect”, the active agents are used to interfere with the

30 apoptosis mechanism in situations where it is detrimental to the health of the

organism. Such situations include, for example, the undesired induction of apoptosis in autoimmune diseases, inflammatory processes and viral or bacterial infections.

Thus, according to this aspect, the present invention provides a pharmaceutical composition for use in the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

- (e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;
- 10 (f) a DNA molecule consisting of a DNA sequence encoding the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2;
- (g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the TAF<sub>II</sub>105 polypeptide of
- 15 (h) the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2; and
- (i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified
- 20 polypeptide retains the normal biological activity of the TAF<sub>II</sub>105 polypeptide encoded by SEQ. ID. NO: 2.

Also provided according to this aspect of the invention is the use of an active agent as defined in (e) – (i) above for the preparation of a pharmaceutical composition for use in the treatment of pathological apoptosis of

25 cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, as well as a method for the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections,, comprising administering to a subject in need, an active agent as defined in (e) – (i) above or a composition comprising such

30 agent.

The aforementioned active agents of both aspects of the invention may be administered to an individual by the use of appropriate carriers, which may be selected from a number of such carriers known *per se*. For example, where said active agent is a DNA sequence or a modified DNA sequence, it will typically be administered within the framework of a vector, as known in the art of gene therapy. As will be appreciated, the vector, which may be a plasmid, viral particle, etc., should preferably be constructed so that it will direct the active DNA sequence into the target cells, namely, cells in which apoptosis is to be induced or cells undergoing undesired apoptosis. Furthermore, the vector should also have the necessary promoters so as to induce expression of the DNA sequence. The manner of construction of appropriate vectors including ensuring appropriate targeting and expression control of the inserted DNA sequence, are generally known and the man of the art should have no difficulties, based on the teaching of the present invention and the general knowledge available in the art, to construct the appropriate vectors.

It is obviously also within the reach of the artisan to prepare pharmaceutical compositions where the active agent is a peptide or polypeptide. At times, it may be desired to include a targeting vehicle in the composition so as to target the polypeptide to the cells, which vehicle may, for example, be a liposome having a targeting moiety, e.g. a ligand of the receptor, on its external surface.

As is also known, the targeting of the inventive compositions to the required site within the body, may also be achieved by direct injection or infusion of the compositions into the site.

## 25 DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, preferred embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is the DNA sequence encoding the TAF<sub>II</sub>105 polypeptide (SEQ. ID. NO: 1);

Fig. 2 is the deduced amino acid sequence of the TAF<sub>II</sub>105 polypeptide (SEQ. ID. NO: 2);

5 Figs. 3A-3C illustrate the interaction of hTAF<sub>II</sub>105 with p65/RelA, a member of the NF- $\kappa$ B family.

3A. NF- $\kappa$ B members p65/RelA and p50 and unrelated OCT2 proteins were translated *in vitro* and labeled with <sup>35</sup>S-methionine using rabbit reticulocyte lysate. These proteins were used for interaction assay with  
10 immobilized flag-tagged TAF<sub>II</sub>105 as indicated on the top of each lane. As a control, the labeled proteins were incubated with the flag beads. The bound proteins were eluted, resolved on SDS-PAGE and autoradiographed. Input lanes represent 10% of the labeled protein used for the binding reaction. 3B. Binding reaction between <sup>35</sup>S-labeled p65/RelA and the purified N-terminal  
15 fragment of TAF<sub>II</sub>105 (amino acids 1-552) fused to glutathione S-transferase (GST) and bound to glutathione beads (lane 2). As a control, a similar reaction was performed using the same beads bound by GST (lane 3). Input lanes represent 10% of p65 used for the binding assay. 3C. Interaction between  
20 TAF<sub>II</sub>105 and the C-terminal activation domain of p65. The C-terminus of p65 (amino acids 398-551) was expressed as a GST- fusion protein, affinity-purified by glutathione beads and subjected to a binding reaction with *in vitro* translated and <sup>35</sup>S-labeled TAF<sub>II</sub>105 (lanes 1-3) or TAF<sub>II</sub>130 (lanes 4-6). GST-containing beads were used as control in a similar binding reaction. Input lanes represent approximately 10% of the labeled proteins used.

25 Figs. 4A & 4B illustrate the interaction of additional NF- $\kappa$ B rel proteins with TAF<sub>II</sub>105.

4A. *In vitro* translated and <sup>35</sup>S-labeled RelB (lanes 1-3) and c-rel (lanes 4-6) were used for an *in vitro* binding assay with the N-terminus of TAF<sub>II</sub>105 similar to Fig. 3 B. 4B. The C-termini of human c-rel (amino acids 302-614)

and mouse relB (amino acids 348-558) were expressed as fusion with GST protein, purified by glutathione beads and used for binding assay with *in vitro* translated and  $^{35}\text{S}$ -labeled TAF $_{\text{II}}$ 105 (lanes 1-4) or TAF $_{\text{II}}$ 130 (lanes 5-8). Input lanes represent 10% of the labeled proteins used in the assay.

5       **Figs. 5A-5D** illustrate the stimulation of p65 and TNF- $\alpha$  induced NF- $\kappa$ B transcriptional activity by TAF $_{\text{II}}$ 105.

5A. Human 293A cells (in 24-well plate) were cotransfected with NF- $\kappa$ B-dependent luciferase reporter plasmid (50 ng) together with either an empty expression vector or the following expression plasmids: 500 ng of  
10 TAF $_{\text{II}}$ 105 (columns 2, 4, 7, 8 and 10); 12.5 ng of p65 (columns 5 and 7); 25 ng of p65 (columns 6 and 8) and 500 ng of I $\kappa$ B $\alpha$  (columns 3, 4, 9 and 10). The amount of CMV-derived vector in each transfection assay was kept constant.  
5B. Similar transfection experiment using HeLa cells. In this experiment the amount of DNA used was 10-fold higher, keeping the relative amount of each  
15 plasmid similar. 5C. An NF- $\kappa$ B luciferase reporter plasmid was cotransfected into 293A cells using similar transfection conditions as in A, with either an empty expression vector (columns 1, 3, 7), a TAF $_{\text{II}}$ 105 expression plasmid (columns 2,5,6, and 8) or with a constitutively active I $\kappa$ B $\alpha$  (lanes 7 and 8). 12 hours post-transfection, 0.15 ng/ml (columns 3 and 4) or 1.5 ng/ml (columns  
20 5-8) of TNF- $\alpha$  were provided to the cells, and 24 hours after transfection, luciferase activity was determined. D. Similar transfection experiment in HeLa cells using 0.15 ng/ml TNF- $\alpha$ .

**Figs. 6A-6C** illustrate the inhibition of NF- $\kappa$ B transcription by dominant negative mutant of TAF $_{\text{II}}$ 105 (TAF $_{\text{II}}$ 105 $\Delta$ C).

25       **6A.** Schematic representation of TAF $_{\text{II}}$ 105 putative functional domains and the dominant negative mutant of TAF $_{\text{II}}$ 105 (TAF $_{\text{II}}$ 105 $\Delta$ C). NLS stands for nuclear localization signal. Expression of mutated TAF $_{\text{II}}$ 105 was confirmed by Western blot (data not shown). **6B.** Luciferase reporter plasmids driven by two tandem NF- $\kappa$ B sites (lanes 1-3), two mutated NF- $\kappa$ B sites (lanes 4 and 5) or a  
30 CMV enhancer (lanes 6 and 7) were transfected into 293A cells together with

the following plasmids: empty expression vector (lanes 1, 4 and 6), with TAF<sub>II</sub>105 $\Delta$ C expression plasmid (lanes 2, 5 and 7) or dominant negative mutant of I $\kappa$ B $\alpha$  (lane 3). 6C. An NF- $\kappa$ B reporter plasmid (50 ng) was transfected into 293A cells together with an empty expression vector (lane 1), 25 ng of p65/RelA expression plasmid (lanes 2-4) and increasing amounts of TAF<sub>II</sub>105 $\Delta$ C expression plasmid (100 and 250 ng, lanes 3 and 4, respectively).

**Figs. 7A-7C** illustrate how a dominant negative mutant of TAF<sub>II</sub>105 induces cell death in response to TNF- $\alpha$ .

**7A.** 293T cells were transfected with wild-type TAF<sub>II</sub>105 (pictures 1 and 2) or TAF<sub>II</sub>105 $\Delta$ C (3 and 4), and either untreated (1 and 3) or treated with 15 ng/ml TNF- $\alpha$  (2 and 4). The pictures were taken 24 hours after transfection.

**7B.** Genomic DNA analysis of 293T cells that were transfected with either an empty expression vector (lanes 1 and 2), TAF<sub>II</sub>105 $\Delta$ C (lanes 3 and 4) or with wild-type TAF<sub>II</sub>105 (lanes 5 and 6). Twelve hours after transfection some of the transfected cells were treated with TNF- $\alpha$  (odd number lanes), and 24 hours after transfection DNA was extracted from the transfected cells and analyzed by 1.8% agarose gel. M stands for a 1 kb DNA size marker. **7C.** Cell survival assay. To quantify the apoptosis induction, 293T cells were cotransfected with a pCMVlacZ reporter plasmid and the indicated expression vectors, and similar to the experiment described in (B), were treated with TNF- $\alpha$  (lanes 2, 4 and 6). 24 hours after transfection the cells were stained with X-Gal, and the numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

**Figs. 8A-8C** illustrate the effect of TAF<sub>II</sub>105 anti-sense RNA expression on TNF- $\alpha$  treated cells.

**8A.** 293T cells were transfected either with empty vector or with expression vector for TAF<sub>II</sub>105 anti-sense RNA. Thirty-six hours after transfection the levels of endogenous TAF<sub>II</sub>105 were analyzed by Western blot using affinity-purified anti-TAF<sub>II</sub>105 antibodies (upper panel). As a control the same extracts were used for analysis of TBP levels (lower panel). **8B.**



Cell-survival analysis of cells expressing TAF<sub>II</sub>105 anti-sense RNA. 293T cells were cotransfected with CMV-GFP reporter plasmid with either an empty expression vector (lanes 1 and 2) or with expression vector for TAF<sub>II</sub>105 anti-sense RNA (lanes 3 and 4). Twenty-four hours after transfection TNF- $\alpha$  was provided to the cells (lanes 2 and 4) and 24 hours later green fluorescent cells were counted in five randomly chosen fields. These data represent an average of four independent transfection experiments. **8C.** DNA fragmentation analysis of cells transfected with either anti-sense TAF<sub>II</sub>105 RNA (lanes 1 and 2) or empty vector (lanes 3 and 4). Twenty-four hours after transfection TNF- $\alpha$  was provided to the cells (lanes 1 and 3) and 16 hours later fresh medium containing TNF- $\alpha$  was provided to the cells. Genomic DNA was extracted 48 hours after transfection and analyzed by 1.8% agarose gel. M represents a 1 kB DNA molecular size marker.

**Figs. 9A-9C** illustrate how a dominant negative mutant of TAF<sub>II</sub>105 inhibits activation of anti-apoptotic genes by NF- $\kappa$ B.

**9A.** 293T cells were transfected with the indicated expression plasmids. 24-hours after transfection, DNA was extracted from the cells and analyzed by 1.8% agarose gel. **9B.** A representative experiment as described in A. The pictures were taken 24 hours after transfection. 293T cells transfected with p65 are shown in panel 1; cells cotransfected with wild-type TAF<sub>II</sub>105 and p65 are shown in panel 2; and cells cotransfected with TAF<sub>II</sub>105 $\Delta$ C and p65 are shown in panel 3. Pictures of cells transfected with either TAF<sub>II</sub>105 or TAF<sub>II</sub>105 $\Delta$ C alone are shown in Fig. 7. **9C.** 293T cells were cotransfected with pCMVlacZ reporter plasmid and expression vectors as indicated in the figure. Twenty-four hours after transfection the cells were stained with X-Gal. The numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

**Figs. 10A & 10B** schematically illustrate a model for the role of TAF<sub>II</sub>105 (TFIID) in activation of TNF- $\alpha$ /NF- $\kappa$ B induced anti-apoptotic genes in 293 cells;

Fig. 11 is a schematic representation of various deletion mutants of the TAF<sub>II</sub>105 N-terminal domain: amino acid 1-552, 1-452, 1-359, 1-167 and 443-452, and their ability to bind p65 and their effect on the survival of p65 expressing cells.

- 5        Fig. 12A shows the structure of a TAF<sub>II</sub>105 $\Delta$ C transgene. The DNA used to generate TAF<sub>II</sub>105 $\Delta$ C transgenic animals contains a cDNA fragment encoding for the N-terminus (amino acid 1-552) of TAF<sub>II</sub>105, an in frame nuclear localization signal (NLS) and influenza virus hemagglutinin tag (HA).  
12B. Southern blot showing the integration of the transgene into the genome of  
10 mice. Restriction enzyme-digested tail DNA of several founders was analyzed for the integration of TAF<sub>II</sub>105 $\Delta$ C by Southern blot using TAF<sub>II</sub>105 $\Delta$ C as a probe. PC is the positive control.

## DETAILED DESCRIPTION OF THE INVENTION

### MATERIALS AND METHODS

#### 15 TAF<sub>II</sub>105

TAF<sub>II</sub>105 was prepared as previously described (Dikstein *et al.*, 1996). The DNA and amino acid sequences are shown in Figs. 1 and 2, respectively.

#### 20 In vitro binding experiments

- Baculovirus expression of flag-tagged TAF<sub>II</sub>105 was carried out as previously described (Dikstein *et al.*, 1996). <sup>35</sup>S-labeled p65, OCT2, and p50 were synthesized *in vitro* by T7 RNA polymerase and rabbit reticulocytes lysate and incubated with flag beads or with TAF<sub>II</sub>105 coupled to flag beads in 0.1M  
25 KCl HEMG buffer (20 mM Hepes pH 7.9, 100 mM KCl, 12.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.1% NP-40, 1mM DTT, 0.2mM PMSF) for 2 hours at 4°C. The beads were washed 3 times with the same buffer and 2 times with 0.2M NaCl HEMG buffer. The bound proteins were eluted by 5 minutes boiling in protein sample buffer followed by SDS-PAGE and autoradiography. The c-rel protein

was translated *in vitro* using T7 RNA polymerase. RelB was synthesized by T3 RNA polymerase. Binding reactions with GST-bound proteins were done in a similar way.

## 5 Plasmids

The baculovirus expression vector for TAF<sub>II</sub>105 was previously described (Dikstein *et al.*, 1996). CMV-TAF<sub>II</sub>105 was constructed by inserting a NcoI-BglIII (NcoI was filled in by the Klenow enzyme) fragment containing TAF<sub>II</sub>105 cDNA and HA Tag from pVLHA-TAF<sub>II</sub>105 (Dikstein *et al.*, 1996) into SmaI-BamHI site of the pCGN vector (Tanaka and Herr, 1990).

To generate the TAF<sub>II</sub>105 $\Delta$ C expression vector, an N-terminal fragment of TAF<sub>II</sub>105 (amino acid 1-552, Dikstein *et al.*, 1996) that was generated by PCR together with an HA tag was first cloned in a CMV-nuc vector (a gift from Dr. Paz Einat) in frame with a nuclear localization signal (NLS). Next, a PstI-HindIII fragment containing NLS, HA and TAF<sub>II</sub>105 $\Delta$ C was inserted into PstI-HindIII sites BlueScript KS+ vector. This fragment was then removed by SmaI and HincII and cloned in a SmaI site of pCGN. The vector for directing anti-sense TAF<sub>II</sub>105 RNA expression contains the same insert, albeit in opposite orientation. Expression of both wildtype and mutant TAF<sub>II</sub>105 after transient transfection was verified by Western blot using anti-HA antibodies (data not shown).

GST-TAF<sub>II</sub>105 $\Delta$ C was generated by cloning an NdeI-EcoRI fragment from pET-TAF<sub>II</sub>105 $\Delta$ C (Dikstein *et al.*, 1996) into NdeI-EcoRI of pGEX-2TKN. The pT $\beta$ STOP-c-rel that was used for *in vitro* translation of c-rel protein was constructed by insertion of an NcoI-EcoRI fragment containing c-rel cDNA (a gift from Dr. Nancy Rice) into NcoI-EcoRI sites of the pT $\beta$ STOP vector. GST-p65C was constructed by inserting an NcoI-XhoI fragment (corresponding to amino acids 398-551) from pET-p65 into NcoI-SalI of pGEX-2TKN. To generate GST-c-relC, a fragment corresponding to amino acids 302-614 was cloned into a StuI site of pGEX2TKN. GST-RelBC was

generated by inserting a BglII-BamHI fragment from pBS-RelB (amino acids 348-558, a gift from Dr. Rodrigo Bravo) into a BamHI site of pGEX-2TKN.

The NF- $\kappa$ B dependent reporter plasmids (wild-type and mutant) were constructed by inserting double-stranded synthetic oligonucleotides (see below) containing either two tandem  $\kappa$ B sites or two mutated  $\kappa$ B sites next to a minimal core promoter from the mouse  $\alpha$ -actin promoter (-40 to +80), that was cloned into Hind III site of a promoterless pLuc vector (Altschmied and Duschl 1997).

NF- $\kappa$ B oligonucleotides:

10 5'-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3';

5'-GATCCGGAAGTCCCCTCGGAAAGTCCCTA-3'

Mutated NF- $\kappa$ B oligonucleotides:

5'-AGCTTATCTACTTTCCGAGTCTACTTTCCG-3';

5'-GATCCGGAAGTAGACTCGGAAAGTAGATA-3'.

15

### Propagation and Transfection of Cell Lines

293 and HeLa cell lines were maintained in F12 Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum. Transfections were performed using the standard CaPO<sub>4</sub> method. 293

20 subconfluent cells were transfected in a 24-well multidish using a total of 1  $\mu$ g plasmid; HeLa cells were transfected using 10  $\mu$ g of plasmid/5X10<sup>6</sup> cells in 100mm petri dish. Luciferase activity was determined according to the instructions of the manufacturer (Promega).

Human recombinant TNF- $\alpha$  was purchased from R & D Systems.

25

### Apoptosis assays

For the survival analysis, 293A or 293T cells were cotransfected with a pCMV-lacZ reporter plasmid and different expression vectors and stained with X-Gal (see below) 36 hr or 48 hr after transfection. The number of blue cells was determined by counting five different randomly chosen fields.

30

For the X-Gal assay the cultures were rinsed with PBS, fixed with 1% glutaraldehyde for 15 min, and then stained with 4 ml of X-Gal solution [2 mg/ml X-Gal in DMF, 3.3 mM  $K_3Fe(CN)_6$  and 3.3 mM  $K_4Fe(CN)_6$ ] for 2 hr.

- DNA fragmentation assay was carried out as follows: 293T cells (2X10<sup>6</sup>) were collected 24 hr after transfection, resuspended in 400  $\mu$ l lysis buffer (200 mM Tris pH 8.5, 100 mM EDTA, 1% SDS, and 100  $\mu$ g/ml proteinase K) and incubated overnight at 37 extraction following ethanol precipitation. Pelleted DNA was resuspended in 100  $\mu$ l TE containing 50  $\mu$ g/ml RNase A and incubated for 30 min at 37°C.
- 10 Twenty micrograms of DNA was run on a 1.8% agarose gel in the presence of 0.5  $\mu$ g/ml ethidium bromide.

## EXAMPLES

- I. **TAF<sub>II</sub>105 interacts with the trans-activation domains of NF- $\kappa$ B members**
- To identify TAF<sub>II</sub>105 target activators, the transcription factors NF- $\kappa$ B and OCT2 that are involved in B cell specific transcription were tested as to whether they could interact with TAF<sub>II</sub>105. For this purpose, recombinant TAF<sub>II</sub>105 was produced in Sf9 cells as a fusion with the flag epitope-tag and purified by anti-flag antibodies coupled to agarose beads. Immobilized TAF<sub>II</sub>105 was incubated with *in vitro* translated and <sup>35</sup>S-labeled p50 and p65 (relA) subunits NF- $\kappa$ B family and OCT2.

- As shown in Fig. 3A, p65 but not p50 or OCT2 specifically and efficiently interacts with TAF<sub>II</sub>105. To determine whether the interaction between p65 and TAF<sub>II</sub>105 is directed by the putative N-terminal coactivator domain of TAF<sub>II</sub>105, this region was expressed in *E. Coli* as a fusion with glutathione S-transferase (GST105 $\Delta$ C) and subjected to a binding reaction with <sup>35</sup>S-labeled p65. As shown in Fig. 3B, p65 specifically binds to the N-terminus of TAF<sub>II</sub>105. We also tested other members of the NF- $\kappa$ B family (c-rel and

RelB) for interaction with TAF<sub>II</sub>105. c-rel but not RelB binds to TAF<sub>II</sub>105N in this assay (Fig. 4A).

Previous studies of activator-TAF association established that an interaction between coactivator subunits of TFIID and transcriptional activators is directed by the trans-activation domain of the activator (Verrijzer and Tjian, 1996). Both p65 and c-rel contain a strong activation domain located at the carboxy-terminus (reviewed by Schmitz and Baeuerle, 1995). To determine the involvement of these activation domains in TAF<sub>II</sub>105 binding, we constructed plasmids for expression of the p65, c-rel and RelB C-termini as fusion with glutathione S-transferase (GST-p65C, GST-RelBC and GST-c-relC). These proteins were purified and immobilized on glutathione Sepharose beads and used in binding assays with an *in vitro* translated and <sup>35</sup>S-labeled TAF<sub>II</sub>105. To determine the specificity of the interaction, a similar binding reaction was also performed with h TAF<sub>II</sub>130, a highly related homologue of TAF<sub>II</sub>105 (Dikstein *et al.*, 1996). The C-terminal activation domains of both p65 and c-rel specifically bind TAF<sub>II</sub>105 but not TAF<sub>II</sub>130 (Figs. 3C and 4B). In this assay, the activation domain of RelB weakly interacts with TAF<sub>II</sub>105 (Fig. 4B).

## II. NF- $\kappa$ B dependent transcription is stimulated by TAF<sub>II</sub>105

To determine the functional importance of TAF<sub>II</sub>105-NF- $\kappa$ B interaction, we tested the effect of TAF<sub>II</sub>105 on transcriptional activation by p65 in transient transfection experiments. A reporter plasmid containing two NF- $\kappa$ B elements upstream to a minimal core promoter and a luciferase gene was cotransfected with p65 and TAF<sub>II</sub>105 into 293 cells. As shown in Fig. 5A, p65 activates NF- $\kappa$ B reporter in a dose-dependent manner (columns 5 and 6). When TAF<sub>II</sub>105 was also cotransfected, both the basal NF- $\kappa$ B and p65 dependent activities were stimulated 2, 5 and 6 fold respectively (columns 2, 7 and 8). Likewise, TAF<sub>II</sub>105 potentiates NF- $\kappa$ B dependent transcription in HeLa cells (Fig. 5B).

To determine whether the activation by TAF<sub>II</sub>105 requires the presence of NF- $\kappa$ B proteins, a similar experiment was carried out in the presence of I $\kappa$ B $\alpha$ , a specific inhibitor of NF- $\kappa$ B factors that prevents the translocation of NF- $\kappa$ B into the nucleus (reviewed in Verma *et al.*, 1995; Baldwin, 1996; Baeuerle and Baltimore, 1996). As expected, I $\kappa$ B $\alpha$  inhibits both constitutive and p65 induced NF- $\kappa$ B activity (Fig. 5A, columns 3 and 9 respectively). In the presence of I $\kappa$ B, no induction of luciferase activity by TAF<sub>II</sub>105 is observed (Fig. 5A, columns 4 and 10).

NF- $\kappa$ B activity is regulated by a broad range of cytokines and external stimuli. TNF- $\alpha$  is among the physiological inducers of NF- $\kappa$ B transcriptional activation. To test whether TAF<sub>II</sub>105 can stimulate TNF- $\alpha$  induced genes, the NF- $\kappa$ B reporter plasmid was transfected into 293 and HeLa cells in the presence or absence of a TAF<sub>II</sub>105 expression plasmid. When the cells are stimulated with sub-optimal doses of TNF- $\alpha$  (0.15 and 1.5 ng/ml), TAF<sub>II</sub>105 stimulates the NF- $\kappa$ B dependent reporter in response to TNF- $\alpha$  (Figs. 5C and 5D). Here again, in the presence of I $\kappa$ B no induction of NF- $\kappa$ B reporter by TAF<sub>II</sub>105 is observed (Fig. 5C, columns 7 and 8). Interestingly, when optimal doses of TNF- $\alpha$  are provided to the cells (10-15 ng/ml), or when the cells are transfected with high amount of p65 expression plasmid, cotransfected TAF<sub>II</sub>105 has little or no additional effect on the NF- $\kappa$ B dependent reporter plasmid that is stimulated to its maximal potential (data not shown) possibly because the basal level of TAF<sub>II</sub>105 present in the cells is sufficient for transcriptional activation when NF- $\kappa$ B concentration within the nucleus is high. These experiments are representative of 3-5 independent transfection experiments with similar results. Expression of transfected TAF<sub>II</sub>105 was verified by Western blot (data not shown).

To further confirm that TAF<sub>II</sub>105 is involved in NF- $\kappa$ B activity a eukaryotic expression vector for a dominant negative mutant of TAF<sub>II</sub>105 was constructed based on its predicted function domains (Dikstein *et al.*, 1996). This mutant is a truncated form of h TAF<sub>II</sub>105 (TAF<sub>II</sub>105 $\Delta$ C, amino acids 1-552,

Fig. 6A), containing the NF- $\kappa$ B binding region but not the TFIID interaction region. Therefore, if TAF<sub>II</sub>105 $\Delta$ C binds NF- $\kappa$ B *in vivo*, it is likely to inhibit NF- $\kappa$ B dependent transcription.

To test the effect of TAF<sub>II</sub>105 $\Delta$ C on NF- $\kappa$ B activity, the NF- $\kappa$ B dependent reporter plasmid was cotransfected with TAF<sub>II</sub>105 $\Delta$ C into 293 cells. The constitutive basal activity of NF- $\kappa$ B was strongly inhibited by TAF<sub>II</sub>105 $\Delta$ C (Fig. 6B). Similarly, cotransfection of the NF- $\kappa$ B reporter with a dominant negative I $\kappa$ B- $\alpha$  inhibited this basal NF- $\kappa$ B activity, confirming that the observed activity is directed by NF- $\kappa$ B proteins (column 3). Reporter plasmids containing either mutation within the NF- $\kappa$ B sites (columns 4 and 5) or the CMV enhancer (columns 6 and 7) were not affected by TAF<sub>II</sub>105 $\Delta$ C further supporting the idea that TAF<sub>II</sub>105 $\Delta$ C is specific to NF- $\kappa$ B and has no effect on core promoter activity.

It was next examined whether a dominant negative mutant of TAF<sub>II</sub>105 can interfere with the trans-activation potential of NF- $\kappa$ B proteins. 293 cells were cotransfected with NF- $\kappa$ B dependent reporter plasmid and p65/RelA transcription factor together with TAF<sub>II</sub>105 $\Delta$ C. As shown in Fig. 6C, TAF<sub>II</sub>105 $\Delta$ C inhibited transcriptional activation by p65/RelA in a dose dependent manner. It is important to note that the amounts of TAF<sub>II</sub>105 $\Delta$ C expression plasmid used in this experiment are insufficient to affect cell viability when cotransfected with p65 (see Fig. 10 below), as evident by microscopic examination and X-gal staining of transfected cells (data not shown).

### 25 III. Inhibition of TAF<sub>II</sub>105 function causes apoptosis in response to TNF- $\alpha$

Recent studies have revealed that activation of NF- $\kappa$ B by TNF- $\alpha$  plays an essential role in protecting cells from pro-apoptotic stimuli produced by TNF- $\alpha$  (Beg and Baltimore, 1996; Wang *et al.*, 1996; Van Antwerp *et al.*, 1996; Liu *et al.*, 1996). Interestingly, when the effect of the TAF<sub>II</sub>105 dominant



negative mutant on long term TNF- $\alpha$  stimulation in 293 cells was analyzed, we observed dramatic changes in 293 cell morphology that are consistent with cell death (Fig. 7A). These morphological changes were not observed in cells transfected with vector alone (data not shown) or with wild type TAF<sub>II</sub>105 (Fig. 7A). The survival rate after TNF- $\alpha$  stimulation in TAF<sub>II</sub>105 $\Delta$ C expressing cells was less than 5% as determined by X-gal staining of transfected cells (Fig. 7C).

To determine whether these changes are related to programmed cell death, 293 cells transiently expressing TAF<sub>II</sub>105 $\Delta$ C were induced by TNF- $\alpha$  and assayed for the DNA ladder characteristic to apoptotic response (Fig. 7B). As expected, no DNA fragmentation appears in TNF- $\alpha$  induced 293 cells transfected with empty expression vector (lane 1). The DNA ladder can be clearly seen only in cells expressing TAF<sub>II</sub>105 $\Delta$ C and stimulated with TNF- $\alpha$  (lane 3), whereas no DNA fragmentation appears in these cells in the absence of TNF- $\alpha$  stimulation (lane 4), or in cells expressing wild type TAF<sub>II</sub>105 in the presence or absence of TNF- $\alpha$  stimulus (lanes 5 and 6).

To further confirm the involvement of TAF<sub>II</sub>105 in activation of anti-apoptotic genes by TNF- $\alpha$ , 293 cells were transiently transfected with expression vector that directs expression of anti-sense TAF<sub>II</sub>105 RNA. As shown in Fig. 8A, transient expression of anti-sense TAF<sub>II</sub>105 reduced the endogenous level of TAF<sub>II</sub>105 protein but not TBP. This reduction is significant considering that although high transfection efficiencies are achieved in these cells, there is still a significant proportion of untransfected or poorly transfected cells that express normal amounts of TAF<sub>II</sub>105 (approximately 20% as determined by X-gal staining after transfection, data not shown). The reduction in TAF<sub>II</sub>105 level is correlated with an increase in cell death in response to TNF- $\alpha$ , as evident by cell survival assay (Fig. 8B), by DNA fragmentation (Fig. 8C) and microscopic examination (data not shown).

#### IV. TAF<sub>II</sub>105 is involved in activation of anti-apoptotic genes by NF- $\kappa$ B

In addition to the role that NF- $\kappa$ B plays in activation of anti-apoptotic genes, numerous reports implicated a role for NF- $\kappa$ B in activation of pro-apoptotic signals (Lee *et al.*, 1995; Lin *et al.*, 1995; Grimm *et al.*, 1996). In 293 cells the involvement of NF- $\kappa$ B proteins in both processes has been documented (Wong *et al.*, 1989; Grimm *et al.*, 1996). When 293 cells are transfected with p65, the cells stay healthy and do not die by apoptosis (Figs. 9A, B and C), presumably because of equilibrium between pro- and anti-apoptotic signals produced by p65. Also, cotransfection of I $\kappa$ B and p65 has no effect on cell survival (Fig. 9A), since I $\kappa$ B prevents NF- $\kappa$ B from entering the nucleus and therefore inhibits any transcriptional activity by p65. Surprisingly, cotransfection of p65 with the dominant negative mutant of TAF $_{II}$ 105 resulted in a strong apoptotic response as evident by DNA fragmentation, the characteristic morphological changes, and surviving cell number (Fig. 9A, B and C respectively). By contrast, no effect is observed with cotransfection of p65 and wild type TAF $_{II}$ 105 (Figs. 9A and B) or by expression of each of these factors alone (Fig. 7). These results confirm the direct role of p65 in activation of cell death in 293 cells and suggest that the TAF $_{II}$ 105 mutant inhibits activation of anti-apoptotic genes but not pro-apoptotic genes by p65. Therefore, it is likely that TAF $_{II}$ 105 is required only for activation of anti-apoptotic genes by NF- $\kappa$ B.

A proposal outlining TAF $_{II}$ 105 activity is summarized in Figs. 10A and 10B. TNF- $\alpha$  activates both anti- and pro-apoptotic cascades (Fig. 10A). The anti-apoptotic pathway requires I $\kappa$ B phosphorylation and degradation followed by NF- $\kappa$ B nuclear localization. In the nucleus, a complex that is formed between NF- $\kappa$ B and TFIID containing TAF $_{II}$ 105 selectively promotes transcription of genes that antagonize the protein synthesis-independent cytotoxic pathway induced by TNF- $\alpha$ . Various agents that can induce NF- $\kappa$ B are also known to stimulate apoptotic signals in cells, such as H $_2$ O $_2$  derived radicals, UV-irradiation, viruses, etc. In 293 cells, activation of NF- $\kappa$ B causes transcriptional activation of both genes that promote apoptosis as well as genes

that inhibit it. The NF- $\kappa$ B-TAF $_{II}$ 105-TFIID complex is selectively engaged in activation of anti-apoptotic genes by NF- $\kappa$ B, while the mechanism of activation of pro-apoptotic genes by NF- $\kappa$ B remains obscure (Fig. 10B).

## 5 V. Preparation of dominant-negative TAF $_{II}$ 105 deletion mutants

On the basis of the results described above, it is possible to inhibit specific functions of native TAF $_{II}$ 105 in a dominant negative manner by using small fragments of the TAF $_{II}$ 105 N-terminus corresponding to different activator binding sites. For example, mapping of NF- $\kappa$ B binding region within TAF $_{II}$ 105 can provide  
10 more efficient and specific inhibitors of NF- $\kappa$ B dependent activation of anti-apoptotic genes. In order to prepare such fragments, it is possible to generate deletion mutants of the TAF $_{II}$ 105 N-terminus. These mutants may be first expressed in *E. Coli* as fusion proteins such as with glutathione S-transferase, and are then used in *in vitro* binding assays with different TAF $_{II}$ 105 target activators such as NF- $\kappa$ B members.

To further investigate the involvement of TAF $_{II}$ 105 in NF- $\kappa$ B induction of anti-apoptotic genes we generated deletion mutants of TAF $_{II}$ 105 $\Delta$ C. Various fragments from TAF $_{II}$ 105 cDNA corresponding to amino acids 1-452, 1-359, 1-167 and 443-552 were generated by restriction enzymes or by PCR. These mutants were expressed in *E. Coli*, purified and analyzed for their ability to bind p65/RelA in an  
15 *in vitro* binding assay with p65/RelA and for their effect on the survival of p65 expressing cells by expressing the fragments in human 293 cells together with p65.

As shown in Fig. 11, TAF $_{II}$ 105 $\Delta$ C (1-552) potently inhibits cell survival (+++) and binds p65 with high affinity (+++). Mutants of TAF $_{II}$ 105 $\Delta$ C deleted of 100 or 193 amino acid residues [105  $\Delta$ C (1-452) and 105 $\Delta$ C (1-359)] have reduced p65  
25 binding capacity (+) as well as reduced inhibitory activity (+) as comwith 105 $\Delta$ C. Similarly, a polypeptide fragment corresponding to amino acid 443-552 is capable of p65 binding and inhibits its anti-apoptotic activity (++) while a fragment (105  $\Delta$ C 1-167) that is unable to bind p65 (-) did not affect cell survival (-).

These findings indicate the existence of two independent p65 binding domains within TAF<sub>II</sub>105 $\Delta$ C and strongly suggest that the direct interaction between TAF<sub>II</sub>105 and p65/RelA plays a role in activation of anti-apoptotic genes.

## 5 VI. TAF<sub>II</sub>105 $\Delta$ C transgenic mice

Another approach which may be taken to study the *in vivo* function of TAF<sub>II</sub>105 is to generate a transgenic mouse model that expresses dominant negative mutants of TAF<sub>II</sub>105 $\Delta$ C. To construct a TAF<sub>II</sub>105 $\Delta$ C transgene, TAF<sub>II</sub>105 cDNA encoding for the non-conserved N-terminus (amino acid 1-552) cloned in pCGN was  
10 used. This is a vector that allows high expression level of a transgene. In addition, an in frame nuclear localization signal and influenza virus hemagglutinin tag (HA) were added to allow nuclear transport of the transgene product and easy detection by anti-HA antibody (Fig. 12A).

This DNA was microinjected into fertilized mouse eggs that were  
15 recovered from superovulated females that had previously mated with males. At the 2-cell stage the embryos were implanted into the oviducts of pseudopregnant foster animals. Using this procedure, several animals were developed from microinjected eggs. These animals were examined for the transgene by Southern blot analysis using total genomic DNA that was prepared from tail biopsies several weeks after birth. As  
20 shown in Fig. 12B, several positive animals in which the transgene has been stably integrated were obtained. The positive animals were mated with wild-type animals. After the birth of the F1 generation, the expression of the transgene mRNA and protein are determined.

To determine whether expression of a dominant negative mutant of  
25 TAF<sub>II</sub>105 has an effect on the transgenic animals, and particularly on the composition of the hematopoietic cell population, the health of transgenic animals of different ages may be compared to non-transgenic wild-type animals. In addition, a histological examination may be performed on spleen, thymus, lymph node, bone marrow, liver and if necessary other tissues. Furthermore, thymus and spleen organs may be  
30 subjected to immunostaining analysis using antibodies directed against different types

of lymphocytes. Subsequently, a cell suspension prepared from hematopoietic tissues such as spleen, thymus and bone marrow may be subjected to FACS analysis using a panel of differentiation markers, and compared to wild-type animals. Both techniques allow the identification of cell populations which are affected by this mutant.

5 It is of particular interest to compare the phenotype of these animals with those of NF- $\kappa$ B, and TNF- $\alpha$  ligand and receptor deficiency. If an affected population is found, the mRNA levels of some tissue/stage specific genes may be measured by Northern blot analysis and compared to those of wild-type animals. mRNAs which will be affected by hTAF<sub>II</sub>105 $\Delta$ C expression are likely to be its target genes (direct or  
10 indirect). Also, primary cells (B or T) from the transgenic animals may be cultured and analyzed for normal cellular processes of this particular tissue, such as transcriptional activation of certain promoters, the specific response to a certain cytokine, etc.

If an *in vivo* effect is found, the region within the TAF<sub>II</sub>105 N-terminus  
15 which confers this effect upon overexpression may be determined. To this end, new transgenic mice may be generated expressing different mutants of TAF<sub>II</sub>105 $\Delta$ C according to the *in vitro* binding assays and the transfection results. The phenotype of the transgenic animals may be determined in a similar manner. It is of particular interest to determine if there is a correlation between the activator binding sites and  
20 the *in vivo* effect, thus linking the biochemical and genetic approaches.

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## CLAIMS:

1. A DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2 or such a modified fragment, wherein said fragment or modified fragment has a dominant negative  
5 • effect on the normal biological activity of said TAF<sub>II</sub>105 polypeptide.
2. A DNA molecule according to Claim 1 wherein said fragment is derived from the N-terminal domain of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2.
3. A DNA molecule according to Claim 2 of SEQ. ID. NO: 1 wherein  
10 said nucleotide sequence encodes a TAF<sub>II</sub>105 $\Delta$ C polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.
4. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a fragment of the TAF<sub>II</sub>105 $\Delta$ C polypeptide having the amino acid sequence selected from the group consisting of amino acids 1-452, 1-359  
15 and 443-552.
5. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the TAF<sub>II</sub>105 $\Delta$ C polypeptide such that the thus obtained modified fragment has a dominant negative effect on the  
20 normal biological activity of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2.
6. An expression vector comprising the DNA molecule of Claim 1 and DNA sequences required for its expression.
7. A polypeptide consisting of a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2 or a modified fragment thereof, wherein said fragment or  
25 modified fragment has a dominant negative effect on the normal biological activity of said TAF<sub>II</sub>105 polypeptide.
8. A polypeptide according to Claim 7 which is a fragment derived from the N-terminal domain of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2.



9. A polypeptide according to Claim 8 of SEQ. ID. NO: 1, which is the TAF<sub>II</sub>105ΔC polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.

10. A polypeptide according to Claim 9 which is a fragment of the  
5 TAF<sub>II</sub>105C polypeptide selected from the group consisting of amino acids 1-452, 1-359 and 443-552.

11. A polypeptide according to Claim 7 which is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the TAF<sub>II</sub>105ΔC polypeptide such that the thus obtained modified fragment  
10 has a dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2.

12. A DNA molecule directing expression of an antisense RNA sequence to SEQ. ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

13. A pharmaceutical composition for inducing an apoptotic process in  
15 pathological cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

(a) a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or more amino acids, wherein the fragment or the modified fragment has a  
20 dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105 polypeptide;

(b) an inhibitor or antagonist of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2;

25 (c) a DNA sequence encoding the fragment or modified fragment of (a); and

(d) a DNA sequence directing expression of an antisense RNA sequence to SEQ. ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

14. A pharmaceutical composition according to Claim 13 wherein the active agent (a) is a polypeptide consisting of a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2 or a modified fragment thereof, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said TAF<sub>II</sub>105 polypeptide.
15. A pharmaceutical composition according to Claim 13 wherein the active agent (c) is a DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2 or such a modified fragment, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said TAF<sub>II</sub>105 polypeptide.
16. A pharmaceutical composition according to Claim 13 for the treatment of cancer.
17. A method for promotion of apoptosis comprising administering to a subject in need, a pharmaceutical composition according to Claim 13.
18. A pharmaceutical composition for treatment of pathological apoptosis of cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:
- (e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;
  - (f) a DNA molecule consisting of a DNA sequence encoding the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2;
  - (g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2;
  - (h) the TAF<sub>II</sub>105 polypeptide of SEQ. ID. NO: 2; and
  - (i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified polypeptide retains the normal biological activity of the TAF<sub>II</sub>105 polypeptide encoded by SEQ. ID. NO: 2.

19. A pharmaceutical composition according to Claim 18 for the treatment of autoimmune diseases, inflammatory processes and viral or bacterial infections.

# **ABSTRACT**

- 5           A DNA molecule comprising a nucleotide sequence encoding a fragment of  
the TAF<sub>II</sub>105 polypeptide subunit of the general transcription factor TFIID, or a  
modified fragment of the polypeptide. The fragment or modified fragment have a  
dominant negative effect on the normal biological activity of the TAF<sub>II</sub>105  
polypeptide. Also disclosed are the polypeptide fragments, as well as pharmaceutical  
10       compositions comprising the DNA molecule or the polypeptide fragment for  
treatment of diseases related to the pathological occurrence of apoptosis.

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GGGACCCCTGGTGACCAAAAGTGGCTCCGGTCAGCGCCCCCTCTAAAGTCAGCA  
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Fig. 1

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Fig. 1 (Con't)

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Fig. 2

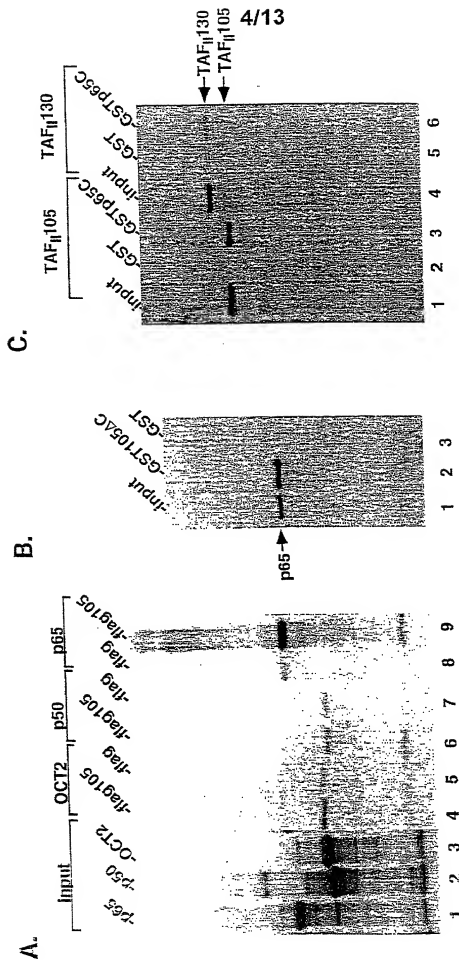


Fig. 3



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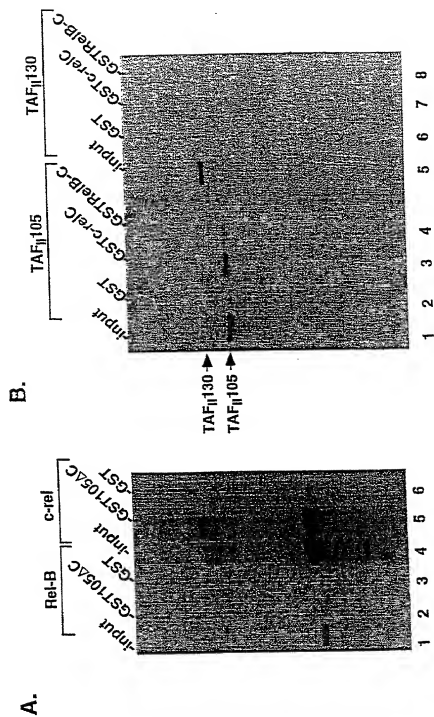


Fig. 4

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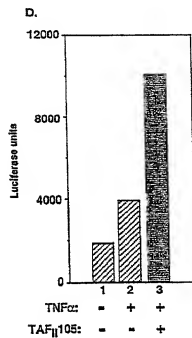
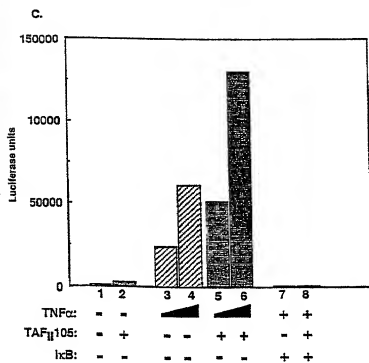
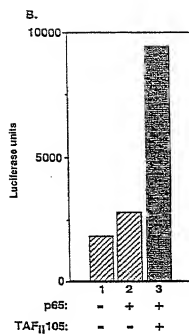
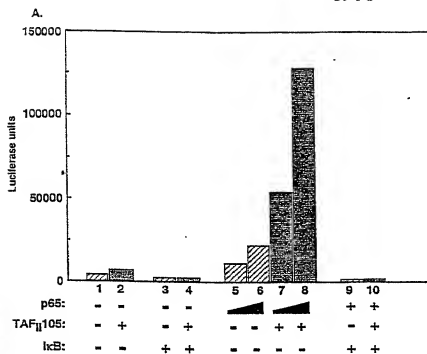
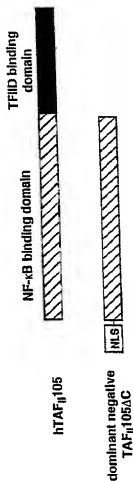
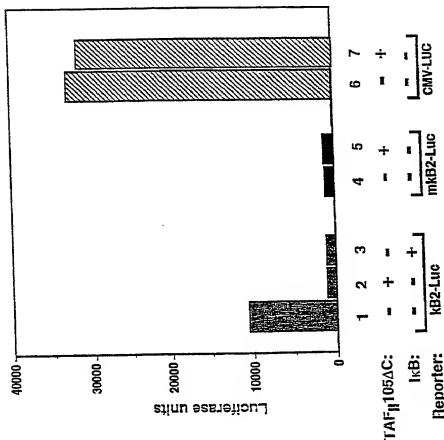


Fig. 5

A.



B.



C.

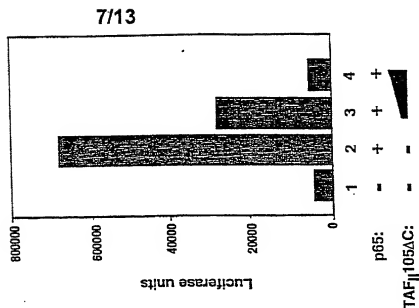
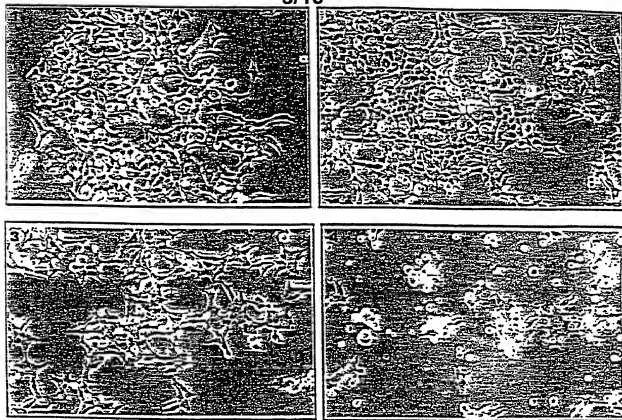


Fig. 6

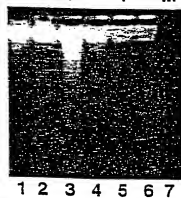
A.

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B.

TAF <sub>II</sub> 105	-	-	-	-	+	+
TAF <sub>II</sub> 105ΔC	-	-	+	+	-	-
TNFα	+	-	+	-	+	-



C.

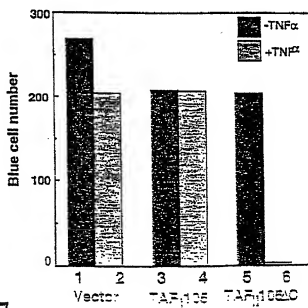


Fig. 7

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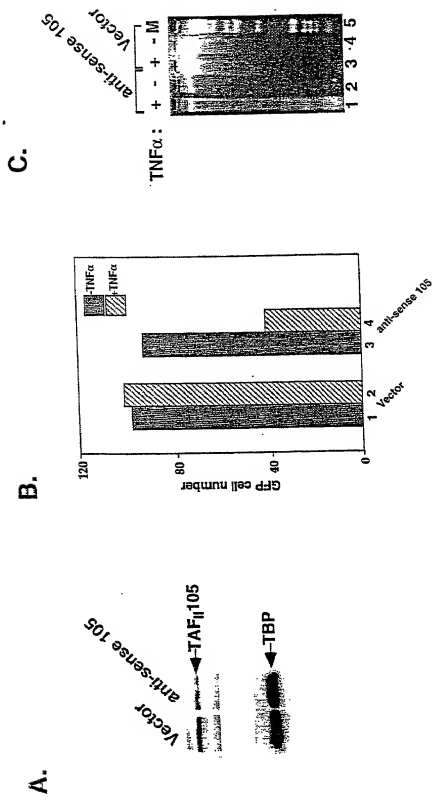
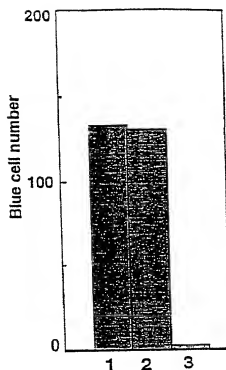
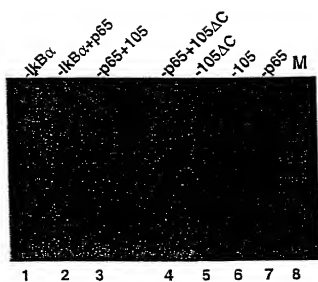


Fig. 8

A.

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C.



p65: + - +  
TAFII105ΔC: - + +

B.

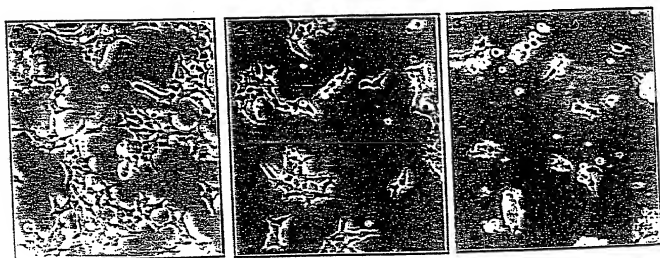


Fig. 9

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B.

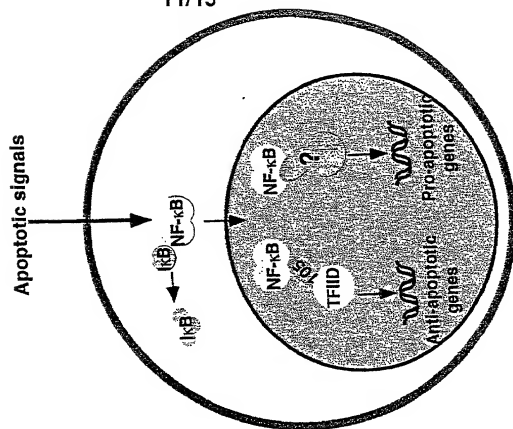
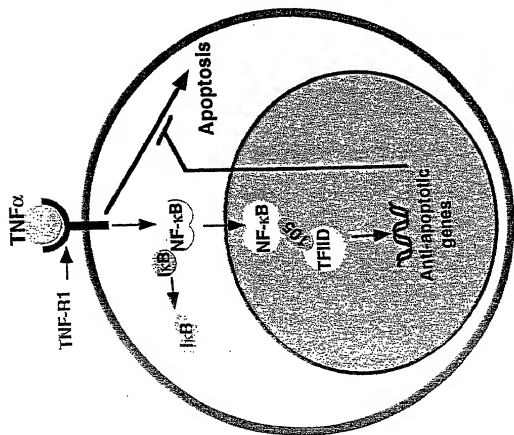


Fig. 10

A.



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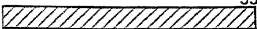
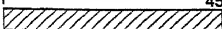
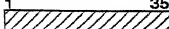


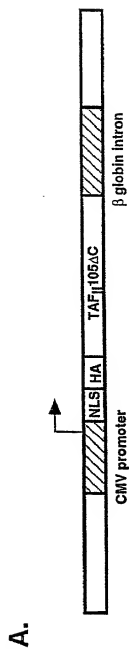
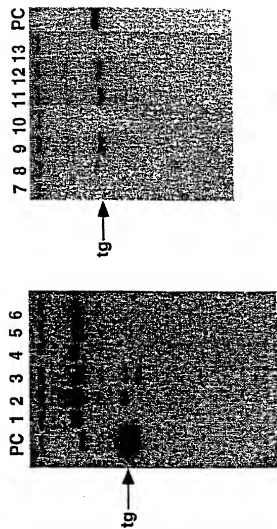
	<u>p65 binding</u>	<u>Cell death</u>
1 552 	+++	+++
1 452 	+	+
1 359 	+	+
1 167 	-	-
443 552 	++	++

Fig. 11



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**B.****Fig. 12**

#26

Attorney's Docket No.: 12204-004001  
 Client's Ref. No.: 131519.1 JP/bv

## COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled A TRANSCRIPTION FACTOR TFIIID SUBUNIT, TAFII105, POLYPEPTIDES, DNA ENCODING THEREOF AND PHARMACEUTICAL COMPOSITIONS, the specification of which:

- ☐ is attached hereto.  
☒ was filed on \_\_\_\_\_ as Application Serial No. **09/763,909** and was amended on \_\_\_\_\_.  
☐ was described and claimed in PCT International Application No. \_\_\_\_\_ filed on \_\_\_\_\_ and as amended under PCT Article 19 on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Serial No.	Filing Date	Status

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
Israel	125971	27 August 1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

### Combined Declaration and Power of Attorney

Page 2 of 2 Pages

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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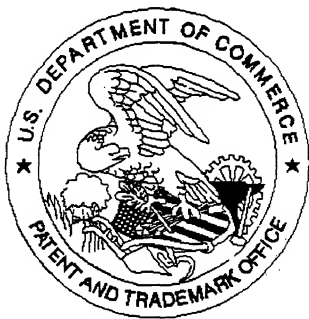
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*Drawing 1.*